

T cell isolation

ST Scott B Thompson JJ Jordan Jacobelli

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 An abbreviated version of this protocol was published in eLIFE in Jun 2020

Formin-like 1 mediates effector T cell trafficking to inflammatory sites to enable T cell-mediated autoimmunity

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Detailed protocol

T cell isolation and purification from secondary lymphoid organs

Jacobelli Lab

1. Retrieve donor mouse and euthanize according to institutional policies;
2. Separately harvest lymph nodes (we typically take inguinal, axillary, brachial, maxillofacial, and mesenteric) and spleen;
3. Place lymph nodes in a sterile mesh filter (40-100um mesh) (e.g. Corning Sterile Cell Strainer) in R10 media (RPMI + 10% FCS + Pen/Strep/Glut + BME) and smash through the filter with the back of a syringe piston;
4. Place the spleen in a separate sterile mesh filter in ammonium chloride (175mM NH₄Cl) and smash through the filter with the back of a syringe piston;
***Note, if you will be using the StemCell EasySep T cell isolation kit in step #7 below you should omit the red blood cell lysis step (#5) since the kit contains a red blood cell depleting antibody. Simply use R10 media instead of ammonium chloride for this step.
5. Lyse red blood cells in the spleen suspension for 3-5 min and then centrifuge (5 min at 400g) and resuspend with R10 media;
6. Count lymph node and spleen cells (you should expect ~100x10⁶ nucleated cells from the spleen and at least 30x10⁶ cells from pooled lymph nodes);
7. To purify CD8 or CD4 T cells use the appropriate negative selection kit from StemCell (e.g. EasySep Mouse CD4+ T Cell Isolation Kit, Catalog # 19852) following the manufacturer's instructions.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Thompson, S. and Jacobelli, J. (2021). T cell isolation. Bio-protocol Preprint. bio-protocol.org/prep1076.
2. Thompson, S. B., Sandor, A. M., Lui, V., Chung, J. W., Waldman, M. M., Long, R. A., Estin, M. L., Matsuda, J. L., Friedman, R. S. and Jacobelli, J.(2020). Formin-like 1 mediates effector T cell trafficking to inflammatory sites to enable T cell-mediated autoimmunity. eLIFE. DOI: [10.7554/eLife.58046](https://doi.org/10.7554/eLife.58046)

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